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Molecular Identification and Antibiotic Resistance Profile of Some *Pseudomonas aeruginosa* Clinical Isolates

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ABSTRACT

Pseudomonas aeruginosa is a Gram-negative, opportunistic bacterium being increasingly recognized as the causative agent of hospital-acquired infection, especially in immunocompromised patients. The bacterium is well known for its environmental persistence and multidrug resistance (MDR). This study aimed to characterize the antibacterial persistence profiles and genetic diversity of *P. aeruginosa* isolates from clinical settings in Sulaymaniyah city, Iraq. Twenty-eight suspected *P. aeruginosa* isolates were collected from hospitals and private laboratories from October 2024 to January 2025. The collected bacteria were identified with standard microbiological procedures, the VITEK 2 system, and confirmation through 16S RNA sequencing. Ten antibiotics were tested following the guidelines of the Clinical and Laboratory Standards Institute for antibiotic susceptibility testing. 12 out of 28 collected isolates were confirmed as *P. aeruginosa*. The antimicrobial susceptibility testing indicated that resistance to Imipenem, Ceftazidime, and Cefepime was seen in 66.7% of the isolates (MDR isolates), while Ceftolozane/Tazobactam had the lowest resistance rate (41.7%). It is observed that 66.7% of isolates subjected to MDR show resistance to three or more antibiotic classes. There is a high prevalence of *P. aeruginosa* in clinical isolates that are resistant to antibiotics. These results underscore the urgent need for improved antimicrobial stewardship programs and the development of alternative treatment options to address this rising public health concern. Through media genomics and molecular methods, reliable identification has been enhanced, which signifies the importance of both studies.

Index Terms: *Pseudomonas Aeruginosa,* Antibiotic Resistance Profile, Multidrug Resistance, 16S rRNA Sequencing, Polymerase Chain Reaction

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, non-fermenting bacterium known for its adaptability and environmental persistence [1]. *P. aeruginosa* has a large genome (5.5–7 Mbp), enabling significant genetic adaptability [2]. This versatility allows the bacterium to thrive in diverse environments, including human, animal, and plant hosts, as well as non-living reservoirs such as water and soil [3], [4]. Despite its environmental persistence, *P. aeruginosa* is rarely found in the normal microbiota of healthy individuals [5]. *This bacterium* is a leading cause of nosocomial infections, contributing to ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and wound infections, particularly in burn and intensive care unit patients [6]–[11]. Its ability to persist in hospital environments makes it a significant public health concern. The increasing antibiotic resistance of *P. aeruginosa* poses a major clinical challenge, and it employs multiple resistance mechanisms, including intrinsic

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resistance (low outer membrane permeability), acquired resistance (horizontal gene transfer [HGT] and mutations), and adaptive resistance (biofilm formation) [12]–[14]. *P. aeruginosa* pathogenicity is attributed to the synthesis of various virulence factors, including pyocyanin, rhamnolipids, elastase, exotoxin A, phospholipase C, and secretion systems such as the type III secretion system [15]. These factors contribute to persistence, antibiotic resistance, and evasion of host defence mechanisms [16]. As a result, *P. aeruginosa* has developed co-resistance to multiple antibiotics, increasing its potential to cause severe and potentially fatal infections [17], and consequently, it has been classified as one of the three bacterial species on the World Health Organization's list of "priority pathogens," urgently requiring the development of new antibiotics [18].

To ensure uniform resistance classification across laboratories, *multidrug resistance* (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [19]. Identification and understanding of the strain diversity of MDR *P. aeruginosa* is crucial due to its ability to cause serious infections and its increasing resistance to multiple antibiotics.

Although *P. aeruginosa* has been studied on a large scale, there is still limited data on its antibiotic resistance patterns and genetic diversity in clinical settings in Sulaymaniyah, Iraq. A recent study in Sulaimanyah city by Seenaa *et al.*, in 2024 showed a prevalence of antibiotic resistance among *P. aeruginosa* isolated from the hospital environment. The resistance rates among the 26 *P. aeruginosa* isolates were as follows: 23.3% to streptomycin, 13.6% to tobramycin, 22.7% to moxifloxacin, 21.2% to levofloxacin, and 22.7% to norfloxacin, as highlighted by Ali *et al.* [20]. In addition, relying only on phenotypic identification methods can sometimes lead to misidentification. Molecular techniques, such as 16S rRNA sequencing, offer greater accuracy in bacterial classification. This study aims to address this gap by integrating both conventional and molecular identification methods to examine the resistance profiles of *P. aeruginosa* isolates from clinical samples.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of 28 bacterial isolates suspected to be *P. aeruginosa* were collected from Sulaimany Burn, Plastic and Reconstructive Surgery Hospital, Dr. Jamal Ahmad Rashid's pediatric teaching Hospital, and a private medical laboratory in Sulaymaniyah Drastic, Kurdistan region, Iraq, between October 1st, 2024, and January 1st, 2025. These bacteria were isolated from clinical samples initially collected and cultured by certain hospitals and laboratories.

2.2. Isolation and Identification of *P. aeruginosa*

2.2.1. Isolation

To obtain a pure bacterial colony, the collected samples underwent a cultivation process. The isolated bacterial strains were subcultured on a Nutrient agar plate and MacConkey agar (Liofilchem), and all plates were incubated at 37°C for 24 h. For the primary isolation, the suspected non-lactose fermented colonies grown on MacConkey agar medium were cultured on the Cetrimide agar medium (Neogen) at 37°C for 24–48 h, which is the recommended medium for the primary identification and isolation of *P. aeruginosa* [21].

2.2.2. Identification

The isolates were subjected to some physiological and biochemical tests. They were identified using conventional biochemical tests, including the Gram staining, oxidase, motility, catalase, citrate utilization, and triple sugar iron (TSI) agar tests [4], [22]. In addition, the VITEK-2 system was employed to confirm the results of the conventional identification methods [23].

2.3. Molecular Identification

2.3.1. Broth culture preparation and genomic deoxyribonucleic acid (DNA) extraction

To extract genomic DNA from representatives of each bacterial group, a loopful of specific bacteria was inoculated into a 15 mL Falcon tube containing 5 mL nutrient broth. The culture was then incubated with shaking at 150 rpm. for 24 h at 37°C. DNA was extracted using the AddPrep genomic DNA extraction kit (addbio) according to the manufacturer's guidelines. The quantity and purity of the extracted DNA were assessed using a Nanodrop (Thermo Scientific NanoDrop 2000, SN 6113). The extracted DNA was then stored at −20°C. For further analysis, 82 ng of genomic DNA was loaded onto a 1% agarose gel and run for 60 min at 80 V.

2.3.2. Amplification of 16S rRNA by standard polymerase chain reaction (PCR)

A ~1515 bp fragment of 16S rRNA was amplified using a PCR method with a final reaction volume of 30 μL. This included 15 μL of 2X Add Taq Master (Ampliqon), 1 μL (5 pmol) of each forward primer (P1F-TGAAGAGTTTGATCATGGCTCAG) and reverse primer (P1R-TTCCCCTACGGTTACCTTGT) [23], and 1 μL

(40 ng) of genomic DNA. The volume was completed by adding 12 µL of nuclease-free water. The PCR was performed using BIO-RAD and Corbett thermal cyclers, configured as follows for VITEK-2-confirmed samples: initial denaturation at 95°C for 5 min, then 27 cycles of 30 s at 95°C for denaturing, 27 s for annealing at 58°C, 1 min for the extension at 72°C, and 5 min for the final extension at 72°C.

2.3.3. Agarose gel electrophoresis

To verify the presence of the correct PCR amplification, 2 µL of the amplicons were electrophoresed on a 1% agarose gel containing 0.07% EtBr, alongside a 100 bp DNA ladder (Genedrix). The gel was run in 1X Tris-borate-ethylenediaminetetraacetic acid buffer at 80 V for 60 min to confirm the proper amplification of the targeted gene. After the run, the DNA molecules were visualized and photographed using a ultraviolet Gel Imager (SynGene 1409).

2.3.4. Partial 16S rRNA sequencing

The resulting approximately 1515 bp PCR amplicon was sent to Macrogen Inc., a South Korean company, for sequencing using P2R (TCTAATCCTGTTTGCTCCCCA) [24], to include the V4 region.

2.3.5. Quality of the sequenced products

The DNA base assembler program was used for sequence quality analysis and editing. The beginning and end of the sequence were trimmed to evaluate its quality.

3. RESULTS

3.1. Isolation and Identification of *P. aeruginosa*

After isolation, the characteristics of the isolates were examined by culturing them on two media: MacConkey agar and Cetrimide agar. The isolates appeared as non-lactose fermenting colonies, producing greenish-blue colonies. In addition, they emitted a characteristic fishy odor and were identified as Gram-negative rods. Of the 28 samples, only 20 isolates grew on Cetrimide agar, which is a selective medium for the isolation and identification of *P. aeruginosa*. Further confirmation of these isolates was carried out using the biochemical tests and VITEK2 system.

3.2 Biochemical Test for Bacterial Isolates

P. aeruginosa was positive for the oxidase, catalase, and motility tests, shows an alkaline slant and alkaline butt on TSI (no acid or gas production), and is positive for the Simmons citrate test (utilizes citrate) [4]. Of the 20 samples isolated from Cetrimide agar, only 15 isolates were positive for oxidase, catalase, citrate utilization, and motility tests, and also appeared as an alkaline slant and alkaline butt on TSI. These bacteria were further conferred by VITEK2 system.

3.3. VITEK2- System

P. aeruginosa was identified in only 12 out of the 20 isolates, as detailed in Tables 1 and 2. *P. aeruginosa* isolates, denoted by their (A numbers).

The analysis of the 12 *P. aeruginosa* isolates revealed distinct patterns of antibiotic susceptibility, classifying the samples into three categories: MDR, sensitive, and intermediate (mixed resistance). Eight samples (A1, A3, A4, A5, A9, A10, A11, and A12) were identified as MDR, showing resistance to antibiotics from at least 3 different classes [25]. These isolates represent a significant concern due to the limited treatment options available for infections caused by such strains. In contrast, three samples (A2, A6, and A8) were

TABLE 1: VITIK-2 system's result for confirmation of *P. aeruginosa* identification

Sample ID	Expectation	Growth on cetrimide agar	VITEK result
PA1	*P. aeruginosa*	Positive	*P. aeruginosa*
PA2	*P. aeruginosa*	Positive	*Enterobacter cloacae complex*
PA3	*P. aeruginosa*	Negative	
PA4	*P. aeruginosa*	Positive	*Proteus mirabilis*
PA5	*P. aeruginosa*	Negative	
PA6	*P. aeruginosa*	Positive	*P. aeruginosa*
PA7	*P. aeruginosa*	Positive	*P. aeruginosa*
PA8	*P. aeruginosa*	Positive	*P. aeruginosa*
PA9	*P. aeruginosa*	Negative	
PA10	*P. aeruginosa*	Positive	*Morganella morganii*
PA11	*P. aeruginosa*	Negative	
PA12	*P. aeruginosa*	Positive	*Proteus mirabilis*
PA13	*P. aeruginosa*	Negative	
PA14	*P. aeruginosa*	Positive	*P. aeruginosa*
PA15	*P. aeruginosa*	Positive	*Morganella morganii*
PA16	*P. aeruginosa*	Positive	*P. aeruginosa*
PA17	*P. aeruginosa*	Positive	*Escherichia coli*
PA18	*P. aeruginosa*	Positive	*P. aeruginosa*
PA19	*P. aeruginosa*	Positive	*P. aeruginosa*
PA20	*P. aeruginosa*	Positive	*P. aeruginosa*
PA21	*P. aeruginosa*	Positive	*P. aeruginosa*
PA22	*P. aeruginosa*	Positive	*Morganella morganii*
PA23	*P. aeruginosa*	Negative	
PA24	*P. aeruginosa*	Positive	*Enterobacter cloacae complex*
PA25	*P. aeruginosa*	Negative	
PA26	*P. aeruginosa*	Negative	
PA27	*P. aeruginosa*	Positive	*P. aeruginosa*
PA28	*P. aeruginosa*	Positive	*P. aeruginosa*

P. aeruginosa: Pseudomonas aeruginosa

TABLE 2: List of confirmed *Pseudomonas aeruginosa* isolates, identified by A numbers

PA	A
PA1	A1
PA6	A2
PA7	A3
PA8	A4
PA14	A5
PA16	A6
PA18	A7
PA19	A8
PA20	A9
PA21	A10
PA27	A11
PA28	A12

found to be fully sensitive to all tested antibiotics, indicating a favourable susceptibility profile. These isolates are expected to respond well to conventional treatments. Only one sample (A7) displayed a limited resistance pattern, showing a combination of resistance and sensitivity across different antibiotics. Sample A7 exhibited resistance to two antibiotics, but remained sensitive to the remaining antibiotics, showing resistance to 2 classes as outlined in Tables 3 and 4.

3.4. Bacterial Identification at the Molecular Level

3.4.1. Genomic DNA extraction

The bacterial sample number from which the genomic DNA was extracted is indicated by lanes 1–12; M; Genedirex 1 kb DNA marker, C–; negative control that was performed without a DNA template (Fig. 1). The wells contain high molecular weight and genomic DNA with yields of on average 82 µg and average purities of 1.85 at (A260/A280) (Table 5). No degradation was observed in all samples. The purity of the DNA was confirmed by the negative control result.

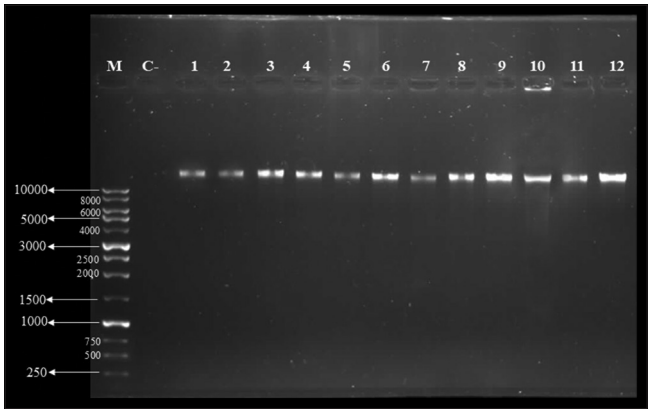

Fig. 1. Agarose gel electrophoresis analysis of genomic deoxyribonucleic acid (DNA) extracted from *Pseudomonas aeruginosa* isolates. From left to right: M is the Genedirex 1 kb DNA marker, C-is the negative control (reaction without DNA template), and lanes 1–12 are the bacterial samples from which genomic DNA was extracted.

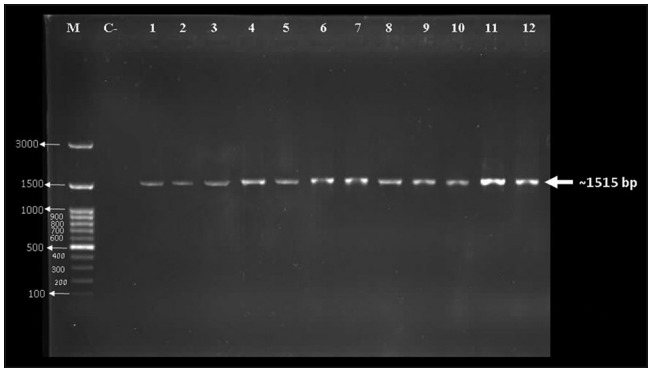

Fig. 2. 16S rRNA partial amplification with P1F and P1R primers. Lanes M and C- represent a 100 bp deoxyribonucleic acid (DNA) marker (Genedirex) and a negative control without a DNA template, respectively. Approximately 1515 bp polymerase chain reaction amplicons produced using DNA templates from A1 through A12 are displayed in Lanes 1–12, respectively.

3.4.2. Amplification of 16S rRNA by standard PCR

The bacterial isolate template DNA was successfully amplified to the expected size of the DNA fragment (~1515 bp), while the negative controls showed no PCR results (Fig. 2).

3.4.3. Partial 16S rRNA sequencing

To identify the species of each bacterial isolate, the reverse primer P2R, which targets the V4 region, was used to sequence the 16S rRNA gene PCR amplicons generated from each isolate using P1F and P1R primers, because the V4 region of the 16S rRNA gene, a hypervariable region, is useful for bacterial identification and phylogenetic analysis because it provides high-resolution differentiation of microbial species. To specify the taxonomic origin of the twelve 16S rRNA sequences, independent computerized alignments were performed versus accessible prokaryotic sequences of 16S rRNA employing EzBioCloud [26]. All of the twelve sequences were highly comparable to reference strains of *P. aeruginosa*, ranging from 99% to 100%. Each isolate had top alignment hits only with *P. aeruginosa*, with query coverage and identity scores compatible with species identification. These molecular results demonstrate that all twelve isolates were *P. aeruginosa* detailed in (Table 6).

3.4.4. Quality of the sequenced products

Depending on the reliability of the DNA sequencing, the 12 *P. aeruginosa* isolates were confirmed to have high-quality values, all exceeding 40, as presented in Table 7 and Fig. 3.

TABLE 3: Antibiotic susceptibility profiles of 12 *Pseudomonas aeruginosa* isolates using the VITEK2 antibiotic panel

Sample ID	VITEK antibiotics panel against *Pseudomonas aeruginosa*									
	AK	GEN	PTZ	IMP	MRP	CIP	CAZ	CPM	CZA	C/T
A1	R	R		R	R	R	R	R	S	R
A2	S	S	S	S	S	S	S	S	S	S
A3	R	R	R	R	R	R	R	R	R	R
A4	R	R	R	R	R	R	R	R	R	R
A5	R	R	R	R	R	R	R	R	S	R
A6	S	S	S	S	S	S	S	S	S	S
A7	S	S	S	R	S	R	S	S	S	S
A8	S	S	S	S	S	S	S	S	S	S
A9	S	S	R	S	S	S	R	R	S	S
A10	R	R	R	R	R	S	R	R	R	R
A11	R	R	R	R	R	R	R	R	R	R
A12	R	R	R	R	R	R	R	R	R	R

R: Resistant, S: Sensitive, I: Intermediate, AK: Amikacin, GEN: Gentamicin, PTZ: Piperacillin/Tazobactam, IMP: Imipenem, MRP: Meropenem, CIP: Ciprofloxacin, CAZ: Ceftazidime, CPM: Cefepime, CZA: Ceftazidime/Avibactam, C/T: Ceftolozane/Tazobactam

TABLE 4: Antibiotic class resistance and susceptibility classification of *Pseudomonas aeruginosa* strains

Sample ID	No. of antibiotics resistant	No. of classes resistant	Susceptibility profile
A1	8	5	MDR
A2	0	0	Sensitive
A3	10	6	MDR
A4	10	6	MDR
A5	9	6	MDR
A6	0	0	Sensitive
A7	2	2	Intermediate
A8	0	0	Sensitive
A9	3	3	MDR
A10	9	5	MDR
A11	10	6	MDR
A12	10	6	MDR

TABLE 5: Quantity and purity of the extracted DNA by nanodrop

	DNA integrity by nanodrop	
Sample ID	A260/A280	Concentration (ng/μL)
A1	1.931	28.0
A2	1.951	28.0
A3	1.861	33.5
A4	1.869	28.5
A5	1.838	34.0
A6	1.841	40.5
A7	1.882	48.0
A8	1.808	47.0
A9	1.862	60.5
A10	1.831	59.5
A11	1.804	46.0
A12	1.809	42.5

DNA: Deoxyribonucleic acid

TABLE 6: Identification of selected isolates by molecular approaches

Sample ID	Suspicion	Per identity (%)	Accession no
A1	*P. aeruginosa*	100	MN606210
A2	*P. aeruginosa*	100	PP515656
A3	*P. aeruginosa*	99.01	CP142446
A4	*P. aeruginosa*	99.58	CP143908
A5	*P. aeruginosa*	99.86	OR452246
A6	*P. aeruginosa*	99.58	ON721331
A7	*P. aeruginosa*	99.73	MG396991
A8	*P. aeruginosa*	100	PP515656
A9	*P. aeruginosa*	100	EF556270
A10	*P. aeruginosa*	99.86	MF144483
A11	*P. aeruginosa*	100	PP515656
A12	*P. aeruginosa*	100	PP515656

P. aeruginosa: Pseudomonas aeruginosa

3.5. Statistical Analysis of Antibiotic Resistance Patterns

According to the level of antibiotic resistance in each bacterial isolate, the bacterial isolates were classified. MDR isolates exhibited resistance to 9.33 distinct antibiotics on average, with a range of 8–10 and a slight variation (standard deviation [SD]: 0.78), as illustrated in Table 8). The susceptible group(s) exhibited no resistance at all, whereas the intermediate-resistant strains were only able to resist two antibiotics (mean: 0.00, SD: 0.00). This analysis demonstrates the differences in how various bacterial strains react to antibiotic therapy. Using a Chi-square test of independence, we found a significant difference in resistance patterns between antibiotics ($\chi^2 = 32.14$, df = 9, $P < 0.001$), indicating that resistance patterns differed considerably between sources (Table 9).

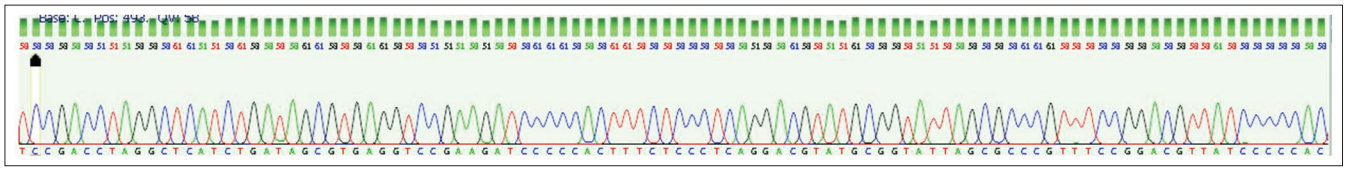

Fig. 3. An example of the 16s rDNA sequence. The quality value of a single base is shown as a numerical value beneath the top green bars. The peaks are evenly spaced, and there is no noise. The baseline remains flat and constant throughout the whole sequence chromatogram. The base is called precisely under each peak (DNA Sequence Assembler v4 (2013), Heracle BioSoft, www.DnaBaser.com) [27].

TABLE 7: Quality value of the sequenced result

Sample ID	Bases left after trimming (%)	Average quality before trimming	Average quality after trimming	Trusted bases (QV >26, %)	Quality remarks
A1	682 (45)	29	55	99.1	Very good
A2	697 (45)	27	56	99.3	Very good
A3	571 (38)	20	42	84.2	Very good
A4	696 (43)	28	53	98.6	Good trust
A5	723 (44)	24	43	92.3	Very good
A6	688 (44)	28	54	98.5	Very good
A7	1159 (94)	35	39	85.6	Good
A8	724 (46)	28	56	99.2	Very good
A9	715 (46)	31	56	99.9	Very good
A10	725 (47)	28	55	98.3	Very good
A11	713 (43)	29	57	99.9	Very good
A12	714 (44)	28	57	100	Very good

TABLE 8: Mean, standard deviation, and range of resistance levels within each susceptibility category (MDR, sensitive, intermediate)

Susceptibility profile	Mean resistant antibiotics	Min	Max	Standard deviation
MDR	9.33	8	10	0.78
Sensitive	0.00	0	0	0.00
Intermediate	2.00	2	2	0.00

MDR: Multidrug resistant

TABLE 9: The Chi-square test to assess the antibiotic resistance differs

Chi-square value	Degrees of freedom (df)	*P*-value
32.14	9	<0.001

3.5.1. Correlation analysis of antibiotic resistance

Using a correlation matrix, the relationships between antibiotic resistance were assessed. Positive correlations (Strong Associations) were found between meropenem (MRP) and imipenem (IMP) (0.92). If a strain demonstrates resistance to one antibiotic, it is highly probable that it will exhibit resistance to other antibiotics as well. Gentamicin (GEN) and Amikacin (AK) (0.87) were strongly linked. Ciprofloxacin (CIP) and Ceftazidime (CAZ) (0.79) were linked to showing resistance as well. CAZ/Avibactam (CZA) showed weak correlations with the other antibiotics, which suggests that CZA might be effective against MDR *P. aeruginosa* that is resistant to other antibiotics (Table 10). Furthermore, to demonstrate the relationships between patterns of antibiotic resistance, a heatmap was generated (Fig. 4).

A strong correlation between AK and GEN (0.87), which reflects shared aminoglycoside resistance mechanisms, and CIP and CAZ (0.79), which may indicate plasmid-mediated co-resistance. MRP and CZA (0.92), which is a striking association that raises concerns about cross-resistance in carbapenem-resistant infections, possibly due to metallo-β-lactamases. However, no correlations (0.00) were observed for other combinations, suggesting independent resistance mechanisms (Fig. 4).

4. DISCUSSION

This study was conducted to assess the prevalence and antibiotic resistance profiles of *P. aeruginosa* isolates from clinical cases in Sulaymaniyah. 20 (71.4%) of the 28 isolates were found to grow on cetrimide agar, a selective medium used for *Pseudomonas aeruginosa* [28]. The results confirmed that although the medium is selective, more accurate techniques such as the VITEK2 automated system and 16S *rRNA* gene sequencing should be employed for identification. Nonetheless, the VITEK2 system only

TABLE 10: Antibiotic resistance correlations among *Pseudomonas aeruginosa* isolates. Strong positive correlations indicate possible co-resistance between specific antibiotic classes, while weak or negative correlations suggest independent resistance patterns

Correlation type	Antibiotic pair	Correlation coefficient (r)	Interpretation
Positive (Strong)	MRP and IMP	0.92	Strong co-resistance to carbapenems
	GEN and AK	0.87	Strong association between aminoglycoside resistance
	CIP and CAZ	0.79	Linked resistance between fluoroquinolone and cephalosporin
Negative or Weak	CZA versus others	Mostly low or negative	Suggests CZA might remain effective against MDR strains

MRP: Meropenemm, IMP: Imipenem, GEN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, CAZ: Ceftazidime CZA: Ceftazidime/Avibactam, MDR: Multidrug resistance

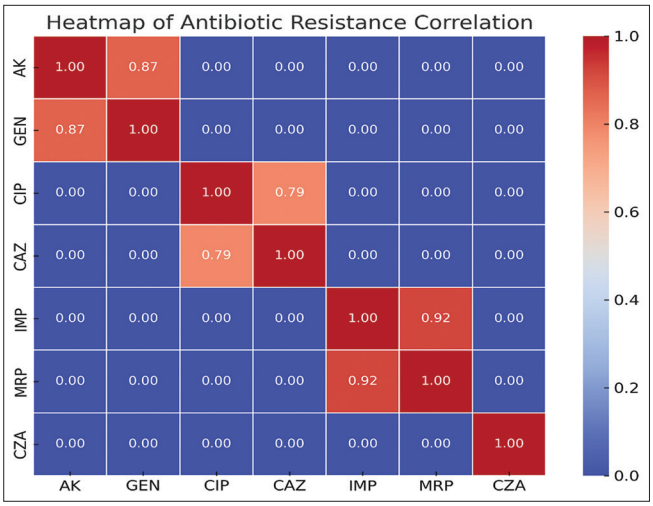

Fig. 4. Heatmap of correlation coefficients between antibiotic resistance patterns among 12 clinical isolates of *Pseudomonas aeruginosa*. The strength of correlation (Pearson's r) is color-coded, with red indicating strong positive correlations and blue indicating weak or no correlation. Notable associations include Meropenem and Imipenem (r = 0.92), and Gentamicin and Amikacin (r = 0.87). Ceftazidime/Avibactam (CZA) showed low or negative correlation with others, indicating potential effectiveness against multidrug-resistant strains.

confirmed 12 as *P. aeruginosa*, indicating a difficulty in the identification of non-fermenting Gram-negative bacteria. Results from the VITEK2 System misidentified many isolates. Possible phenotypic overlap is likely due to similar appearances of *Pseudomonas* spp. and other Gram-negative bacteria, including *Enterobacter cloacae* and *Proteus mirabilis*. This emphasizes the significance of 16S RNA as an identification tool [21], [29], [30]. The differences in identification demonstrated the need for ongoing training and updates on laboratory procedures as bacterial profiles continue to evolve.

The confirmed *P. aeruginosa* isolates showed a tendency towards MDR in the antibiotic susceptibility profiles. Eight MDR isolates were found to be resistant to all (66.7%) and pose a great challenge for antibiotic treatment options. The findings of the current study are aligned with several regional studies that report high levels of MDR. For example, the study by Alkhulaifi and Mohammed reported 72.63% MDR in Basra, Iraq [31]. Similarly, Li *et al.*, reported a rate of 87.5% in Iran and 55.9% in Turkey [32]. As per Al-Orphaly *et al.*, the MDR rates of the neighbouring countries Bahrain (86%), Egypt (75.6%), Lebanon (64.5%), and Jordan (52.5%), were also high. Similar findings indicated that similar causes may exist for the globally common *P. aeruginosa* resistance in the Middle East, such as antibiotic consumer behavior and medical practices [33]. Lower resistance rates were also reported at 17.6% in Thailand, 17.7% in the US, and 22.2% in Croatia [32]. In Europe, the total prevalence estimated of MDR is 29.9 [32]. The fairly low numbers could be a result of more controlled prescription practices as well as better hospital hygiene standards and stronger antibiotic stewardship policies [34]. Statistical analysis supported these findings. Most MDR isolates showed resistance to at least 9 antibiotics. The factors that may be responsible for the high occurrence of antibiotic resistance observed in this study are interrelated. A major contributor to antibiotic resistance is the indiscriminate use of antibiotics, which refers to the overprescribing of these drugs by health care providers and self-medication practices in the community. Excessively using antibiotics forces the bacteria to mutate. Besides, the lack of public knowledge about the proper use of antibiotics–like not finishing the prescribed course or using them for viral infections–is a contributor to the emergence and spread of resistance. The problem is made worse by the suboptimal infection control in the clinical and community settings that favour the transmission of resistant pathogens. The increase in drug resistance, the lack of new antibiotic discoveries, and the worldwide public health burden imposed by bacterial resistance jointly call for antibiotic stewardship programs and education, and monitoring.

The resistance patterns were not randomly distributed, as the Chi-square test results show dependency on the antibiotic class. Carbapenems (MRP and IMP) and aminoglycosides (GEN and AK) showed a high correlation, indicating potential

for the shared mechanism of resistance or common pressure. On the other hand, CAZ/Avibactam's weak correlations with other antibiotics indicate a potential efficacy against MDR strains, allowing for successful treatment. In hospitals, patients are frequently immunocompromised [35], [36]. The resistance patterns or intermediate resistance showed (1 isolate) 8.3% further complicating the treatment regimens [37]. Surprisingly, 3 isolates (%25) exhibited complete sensitivity to all antibiotics, suggesting that not all *P. aeruginosa* are resistant. As for the subject matter experts, this finding is quite important. It shows that effective treatment options are still available for some strains. Thus, the clinicians must focus on precise identification and susceptibility testing. The identification of sensitive strains can assist clinicians in determining the correct choices of therapy. Molecular identification of the isolates using 16S rRNA sequencing gave a firm confirmation of the species and showed genetic diversity among the isolates [38]. This study highlights the importance of molecular methods in their diagnostic application in antibiotic resistance characterization. Testing organisms in culture often do not have the same sensitivity as molecular methods and may miss bacterial pathogens with even high relative abundance. On the other hand, the molecular approach (e.g., 16S *rRNA* gene sequencing) improves the accuracy of identifications. The reliability of these methods is further confirmed by the high percentage of sequence identity with established *P. aeruginosa* reference strains. In addition, it was found that genomic analysis of *P. aeruginosa* is important to elucidate the evolution of infection epidemiology and possible HGT mechanisms involved in the transmission of antibiotic resistance genes [39].

5. CONCLUSION

This study investigated the antibacterial resistance profiles and genetic diversity of Pseudomonas aeruginosa. A notable prevalence of multidrug resistance (MDR) was detected in eight (66.7%) of the isolates, with four isolates (33.3%) exhibiting resistance to all tested antibiotics. Resistance against imipenem, ceftazidime, and cefepime was at its highest level (58.3%), while ceftolozane/tazobactam showed the lowest resistance rate (41.7%). A significant correlation was identified between carbapenems and aminoglycosides, suggesting the involvement of similar resistance mechanisms. Conversely, the weak correlations of CAZ/avibactam with other antibiotics indicated its potential efficacy against MDR strains, which could facilitate successful therapeutic interventions. Furthermore, three isolates (25%) showed complete susceptibility to all antibiotics tested, indicating that not all P. aeruginosa strains are resistant and that viable treatment options remain available for certain strains. Thus, clinicians must prioritise accurate identification and susceptibility testing. This study highlights the necessity for refined methodologies to identify antibiotic resistance, including the VITEK2 automated system and molecular techniques using 16S rRNA gene sequencing.

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